EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 10
84-92
Year: 2017

THE RELATIONSHIP BETWEEN CLINICALLY MANIFEST GASTRODUDENAL DISEASE AND THE GENOTYPE OF THE ASSOCIATED HELICOBACTER PYLORI ORGANISM

Eman Zaki Azzam*[1], Ezzat Ali Ahmed [1], Wafaa Mohammed Hussein Mekky[1], Shwikar Mahmoud Abd El-Salam[2], Essam El Din Abd El Gawad Abd El Khaliq[3]

[1]Internal Medicine Department, Faculty of Medicine, Alexandria University. Egypt.
[2]Microbiology Department, Faculty of Medicine, Alexandria University. Egypt.
[3]West El Nubaria Hospital. Ministry of Health, Egypt.

***Corresponding Author: Eman Zaki Azzam**
Internal Medicine Department, Faculty of Medicine, Alexandria University. Egypt.

Article Received on 06/08/2017 **Article Revised on 26/08/2017** **Article Accepted on 16/09/2017**

ABSTRACT
Infection with H. pylori has been associated with gastroduodenal diseases such as gastritis, gastric ulcer, duodenal ulcer, gastric cancer and mucosa- associated lymphoid tissue lymphoma (MALT lymphoma). The aim of this study was to detect the relationship between clinically manifest gastroduodenal disease and the genotype of the associated H. pylori organism. The study was conducted on 20 control subjects and 100 patients suffering from upper gastro-intestinal symptoms in the form of dyspepsia, epigastric pain, vomiting, heartburn, hematemesis and melena in which infection with H. pylori was detected by monoclonal antibody based H. pylori stool antigen test. Upper gastrointestinal tract (GIT) endoscopy was performed in all subjects as well as samples from gastric antral mucosa were obtained to detect H.pylori by rapid urease test, Polymerase chain reaction (PCR) for detection of H.pylori strain and the presence or absence of its virulence factors. Results showed that CagA and VacA are two virulence factors that can affect the clinical outcome of H.pylori and there was a significant positive association between the presence of CagA+ve strains and gastroduodenal diseases. VacA s1 genotype was associated significantly with gastroduodenal diseases but VacA m1 allele showed insignificant association.

KEYWORDS: H.pylori, CagA, VacA, gastroduodenal diseases, PUD, gastric malignancies.

INTRODUCTION
Helicobacter pylori (H.pylori) is a gram negative spiral shaped microorganism about 3 micrometers (µm) long with a diameter of about 0.5 µm. It is microaerophilic and has 4-6 flagella that make it highly motile.[1]

H. pylori is one of the most common worldwide chronic bacterial infections and is currently estimated that approximately half of the world population is infected with the bacterium. However, the prevalence of H. pylori is not homogeneous, being less prevalent in western countries.[2]

H. pylori colonizes the gastric epithelium of the human stomach[3] early in life (<10 years of age) and remains latent in the majority of infected patients.[4] It becomes symptomatic later in life when the infection results in development of gastroduodenal disease. The frequency and severity of disease outcome differs between infected patients and depends on interplay between the virulence of the infecting strain of the bacterium, the type and severity of the patients' immune response and environmental factors.[5]

H. pylori strains possess specific virulence factors that play an important role in the development of gastritis, peptic ulcer diseases (PUD), gastric adenocarcinoma and gastric mucosa associated lymphoid tissue lymphoma (MALToma).[6] One of these virulence factors is the CagA protein encoded by the cytotoxic associated gene A (CagA) present in the genome of H. pylori. This toxic protein when gains access to gastric epithelial cells becomes active through its phosphorylation on tyrosine residues by the host cell kinase. It stimulates cell signaling pathways leading to cytoskeletal changes, epithelial cell proliferation or cell apoptosis which if not well regulated may lead to malignant diseases or ulcers.[7] It may also disrupt apical junctional complexes between epithelial cells leading to loss of barrier function.[8] Most strains of H. pylori express CagA protein but the protein differs in structure between strains. CagA protein may have between zero and 5 active tyrosine phosphorylation sites. More sites lead to

high levels of CagA phosphorylation in epithelial cells. Strains with more sites are commonly isolated from patients with precancerous gastric changes (atrophy and intestinal metaplasia) as well as patients with gastric cancer.[9]

The other virulence protein is VacA protein encoded by the Vacuolating cytotoxic gene A (VacA) which is present in the genome of all H. pylori strains. The mature secreted VacA protein is polymorphic with variable cytotoxic activity. The toxin is composed of regions; the signal peptide region (s) and the intermediate or binding region (m). The signal peptide region is of two types; s1 or s2, s1 type is active and s2 type is inactive. The binding region is also of two types m1 or m2; m1 type is active and m2 type is less active. VacA protein may comprise any combination of s and m types. Type s1m1 VacA is fully active, type s1m2 is active but binds to narrower range of cells; type s2m1 and s2m2 do not induce Vacuolation.[10-11] VacA s1m1 and s1m2 strains have been associated with peptic ulcer diseases and s1m1 strain has been associated with gastric cancer.[12] The aim of this study was to detect the relationship between clinically manifest gastroduodenal disease and the genotype of the associated H.pylori strain.

MATERIALS AND METHODS

The present study was conducted on 100 Egyptian patients having upper gastrointestinal symptoms and infected with Helicobacter pylori. They were submitted to upper gastrointestinal endoscopy in gastroenterology department of Alexandria university hospital. Also, 20 asymptomatic persons were included as controls.

Selection criteria

Inclusion criteria: Patients with H. pylori infection documented by stool antigen test for H. pylori.

Exclusion criteria

- Patients aged > 70 years old.
- Patients with hepatic failure.
- Patients with renal failure.
- Patients with cardiac failure.
- Patients with bleeding and/or coagulation disorders.
- Patients taking antisecretory drugs.
- Patients receiving anti-Helicobacter pylori antibiotic or bismuth in the prior six months.

All patients stopped any anti-ulcer therapy and antibiotics for at least two weeks before enrollment in this study. An informed consent was obtained from all subjects, and the study was approved by the ethics committee of Alexandria University.

All of the subjects included in the study were subjected to:

1) Full history taking with particular stress on:

- Analysis and duration of the complaint (epigastric pain, heartburn, dyspepsia, vomiting, hematemesis, melena and loss of weight).
- History of smoking, drug intake, and any associated diseases.
- History of any operations and/or procedures and complications if present.

2) Complete clinical examination

3) Laboratory investigations

a- Routine investigations including CBC, fasting blood sugar, renal function tests, liver enzymes, prothrombin time and activity.

b- Detection of Helicobacter pylori by monoclonal antibody based H. pylori stool antigen test.[13]

4) Upper gastrointestinal endoscopy and biopsy

Diagnostic upper gastrointestinal endoscopic examination was done to all subjects, the endoscopic findings were recorded. From these patients, gastric biopsy specimens were taken from the antrum within 2 cm of the pyloric channel for rapid urease test (one tissue biopsy), PCR assay (one tissue biopsy), microbiological examination (three fragments), and histopathological examination (three fragments).

Endoscopic observation and histopathologic confirmation were used to determine patients' pathologies. The benign gastroduodenal diseases were divided into the following general categories: non-erosive gastritis, gastric ulcer disease (erosive gastritis and ulcers) and duodenal ulcer disease (duodenitis and duodenal ulcers), whereas the malignancies included gastric adenocarcinoma and primary gastric lymphoma.

5) Polymerase chain reaction (PCR) examination

DNA extraction

DNA was extracted from biopsy specimens using QIAamp DNA Mini Kit with tissue DNA extraction protocol.The sample was put in 180µl of buffer ATL (QIAGEN) with 20µl of proteinase K and then incubated overnight at 56°C. After lysis of the sample, 200µl of buffer AL was added to the sample and the mixture was incubated for 10 min at 70°C. The mixture was then combined with 200 µl of absolute ethanol and mixed by pulse-vortexing for 15 s. The mixture was applied to a spin column, which holds a silica gel membrane, and spun for 1 min at 6,000 × *g*. The spin column was washed with 500 µl of buffer AW1 and then AW2 by centrifugation at 20,000 × *g* for 1 and 3 minutes respectively. The DNA bound on a membrane was eluted by centrifugation with 50 µl of buffer AE after a 5-min incubation at room temperature. The resulting DNA extracts were stored at - 20°C until PCR assessment.

PCR amplification for detection of H.pylori DNA[14]

The PCR was performed with primers for urease gene ure C (136 bp) 5' –AAGCTTTTAGGGGTGTTAGGGGTTT–3' and 5'–CGCAATGCTTCAATTCTAAATCTTG–3' indicative of H. pylori infection. Amplification was performed in a final volume of 50 µl of PCR mixture containing 0.8 µm of each primer, 10 mM of each deoxy nucleotide

triphosphate (dATP, dGTP, dTTP and dCTP). 10 mM tris HCl, 50 mM KCl. 0.1% triton X– 100. 1.5 mM $MgCl_2$, 1 unit of DNA polymerase (Fermentas) and 10µl of template DNA. DNA amplification was carried out as follows: Denaturation at 94°C for 5 minutes in the first cycle, followed by annealing for 30 seconds at 60°C, extension for 2 minutes at 72°C, and denaturation for 30 seconds at 94°C for a total of 40 PCR cycles. The extension for the last cycle was increased to 5 minutes to ensure complete extension of the amplified fragment. The PCR products were resolved by 2% agarose gel electrophoresis and were visualized after ethidium bromide (0.5µg/ml) staining, using an UV transilluminator and photographed by Polaroid camera.

Multiplex PCR to detect H. pylori CagA and VacA genotypes[15]

PCR was performed to detect CagA and VacA (s1/s2, m1/m2) using primers for CagA, and the signal regions (s1 & s2) and mid regions (m1 & m2) of the VacA gene. Amplification was performed in a final volume of 50 µl of PCR mixture containing 0.5 µM of each primer, 10 mM of each deoxy nucleotide triphosphate (dATP, dGTP, dTTP and dCTP). 10 mM tris HCl, 50 mM KCl. 0.1% triton X– 100. 1.5mM $MgCl_2$, 1 unit of DNA polymerase (Fermentas) and 10 µl of template DNA. DNA amplification was carried out under the following general conditions: 30 cycles of 94° C for 1 min, 55°C for 1 min and 72°C for 1 min. The amplified genes were detected by electrophoresis in a 1.5% agarose gel with ethidium bromide and bands were visualized using an UV transilluminator and photographed by Polaroid camera.

Statistical analysis was performed using SPSS version 18.0. Cross-tabulation, frequency tables and chi-square tests were derived in order to detect any association between different variables under study.

RESULTS

1. Demographic data (table 1)

Table 1: Age and sex distribution among cases and controls.

	Age (Mean ± SD)	Sex No (%)	
		Male	Female
Cases	38.4 ± 12.39	45 (45%)	55 (55%)
Controls	29.5 ± 6.62	11 (55%)	9 (45%)
Test (p-value)	t = 4.61 (<0.001)*		χ^2=0.67 (0.413)

t: T test
χ2: Chi square test
P: probability value
*: Statistically significant at $p \leq 0.05$

2. Clinical data (table 2)

The main complaint was epigastric pain and vomiting in 35 patients (35%) followed by heartburn and dyspepsia in 28 patients (28%), hematemesis in 27 patients (27%) and melena in 10 patients (10%).

Table 2: Clinical presentation of patients.

Clinical presentation	No of patients	%
Epigastric pain &vomiting	35	35%
Heart burn &dyspepsia	28	28%
Hematemesis	27	27%
Melena	10	10%

3. Endoscopic findings (table 3)

The most common endoscopic finding was non-erosive gastritis (n= 54, 54%) followed by peptic ulcer diseases (n= 39, 39%) and gastric malignancy (n=7, 7%). Gastric adenocarcinoma was diagnosed in 5 patients, while lymphoma was diagnosed in 2 patients.

Table 3: Endoscopic findings endorsed by histopathological examination.

Endoscopic findings	Non-erosive gastritis	Peptic ulcer diseases n=39 (39%)		Gastric malignancy n=7 (7%)	
		Gastric ulcer disease	Duodenal ulcer disease	Adenocarcinoma	Lymphoma
NO.	54	26	13	5	2
%	54%	26%	13%	5%	2%

4.Virulence factors of H. pylori

Types of H. pylori (table 4)

By using tissue PCR, the prevalence of CagA and VacA expression to define the type of H. pylori was performed. For that purpose, the strains were categorized into 3 types: type I expressing both CagA and VacA, type II expressed neither CagA nor VacA, type III expressing CagA only. The predominant H. pylori type in our patients was type I (n=53, 53%). The prevalence of type I H. pylori in non-erosive gastritis, peptic ulcer diseases and gastric malignancy was 57.4% (31 of 54), 48.7% (19 of 39) and 42.9 % (3 of 7), respectively. In controls, type II H. pylori was the most common type (n=16, 80%).

Table 4: Types of H. pylori among cases and controls.

	Type I CagA+ve VacA +ve	Type II CagA-ve VacA –ve	Type III CagA+ve only	Total
Cases No(%)	53 (53%)	6 (6%)	41 (41%)	100 (100%)
Controls No (%)	1 (5%)	16 (80%)	3 (15%)	20 (100%)
Test (p-value)	χ^2=61.388 (<0.001)*			

I. CagA status (table 5)

Most of the cases were CagA+ve (94%) while CagA +ve strains were present in 4 of the control subjects (20%). This association was statistically significant (p<0.001).

II. VacA status (table 5)

53 patients were VacA +ve (53%) while only one control subject was VacA +ve (5%). There was a significant association between the VacA positivity and the disease status(p<.001).

Table 5: CagA and VacA status among cases and controls.

	CagA		VacA	
	+ve	-ve	+ve	-ve
Cases No (%)	94 (94%)	6 (6%)	53 (53%)	47 (47%)
Controls No (%)	4 (20%)	16 (80%)	1 (5%)	19 (95%)
Test (p-value)	χ^2=60.96 (<0.001)*		χ^2= 15.515 (<0.001)*	
OR (95%CI)	62.67 (15.99-247.04)		21.43 (2.76-166.24)	

χ2: Chi square test
P: probability value
*: Statistically significant at $p \leq 0.05$
OR: Odds ratio
CI: Confidence interval

III Relationship between virulence factors and non-erosive gastritis comparing cases and controls (table 6).

In comparison between cases and controls, there was a significant positive association between H. pylori virulence factors (CagA and VacA) and non-erosive gastritis. For CagA, (FEP<0.001, OR=104, 95%CI=17.41, 621.33), while for VacA, (p<0.001, OR=25.61, 3.19-205.39).

Table 6: Relation between virulence factors and non-erosive gastritis comparing cases and controls.

	CagA		VacA	
	+ve	-ve	+ve	-ve
Cases with non-erosive gastritis No (%)	52 (96.3%)	2 (3.7%)	31(57.4%)	23 (42.6%)
Controls No (%)	4 (20%)	16 (80%)	1 (5%)	19 (95%)
Test (p-value)	Fisher's Exact Test (FEP<0.001)*		χ^2= 16.33 (<0.001)*	
OR (95%CI)	104, (17.41, 621.33)		25.61 (3.19-205.39)	

χ2: Chi square test
P: probability value
*: Statistically significant at $p \leq 0.05$
OR: Odds ratio
CI: Confidence interval
FEP: Fisher's Exact p value.

IV. Relationship between virulence factors and PUD comparing cases and controls (table 7)

It was noticeable that the virulence factors (CagA and VacA) were associated significantly with PUD when comparing cases and controls. As regards CagA, (P<0.001, OR=35, 95%CI=7.76, 157.94), while the values for VacA were (p<0.001, OR=18.05, 2.19-148.38).

Table 7: Relation between virulence factors and PUD comparing cases and controls.

	CagA		VacA	
	+ve	-ve	+ve	-ve
Cases with PUD No (%)	35 (89.7%)	4 (10.3%)	19 (48.7%)	20 (51.3%)
Controls No (%)	4 (20%)	16 (80%)	1 (5%)	19 (95%)
Test (p-value)	χ^2=28.69 (<0.001)*		χ^2= 11.28 (<0.001)*	
OR (95%CI)	OR=35, (7.76, 157.94)		18.05, (2.19, 148.38)	

χ2: Chi square test
P: probability value
*: Statistically significant at $p \leq 0.05$
OR: Odds ratio
CI: Confidence interval

V. Relationship between virulence factors and gastric malignancies comparing cases and controls (table 8)
There was a positive significant relationship between the virulence factors (CagA and VacA) and gastric malignancy. For CagA, (FEP<0.001, OR=0.2, 95%CI: 0.083, 0.481), while for VacA, (FEP =0.042, OR= 14.25, 95%CI: 1.16, 174.8).

Table 8: Relation between virulence factors and gastric malignancy comparing cases and controls.

	CagA		VacA	
	+ve	-ve	+ve	-ve
Cases with gastric malignancy No (%)	7 (100%)	0 (0.0%)	3 (42.9%)	4 (57.1%)
Controls No (%)	4 (20%)	16 (80%)	1 (5%)	19 (95%)
Fisher's Exact Test (p-value)	FEP <0.001*		FEP =0.042*	
OR (95%CI)	0.2, (0.083, 0.481)		14.25, (1.16, 174.8)	

FEP : Fisher's Exact probability value
*: Statistically significant at $p \leq 0.05$
OR: Odds ratio
CI: Confidence interval

VI. VacA s genotype in relation to endoscopic findings among patients (table 9)
The most common VacA genotype among patients was s2m1 (41.51%, 22 of 53), followed by s2m2 (22.64%, 12 of 53), then s1m1 (18.87%, 10 of 53) and s1m2 (16.98%, 9 of 53).

Table 9: VacA genotypes and the associated gastroduodenal disease among patients.

VacA genotyping		Endoscopic findings			Total
		Gastric malignancy	Non-erosive gastritis	peptic ulcer diseases	
s1m1	No.	2	3	5	10
	%	66.67%	9.68%	26.16%	18.87%
s1m2	No.	1	2	6	9
	%	33.33%	6.65%	31.58%	16.98%
s2m1	No.	0	17	5	22
	%	0.0%	54.84%	26.16%	41.51%
s2m2	No.	0	9	3	12
	%	0.0%	29.03%	15.79%	22.64%
Total	No.	3	31	19	53
	%	100.0%	100.0%	100.0%	100.0%

VII. Relation of VacA s genotypes and endoscopic findings (table10)
There was a significant association between s1 allele and gastric malignancy (OR=33.73, 95% CI=1.52, 750.35, P=0.010).

Table 10: Relation of VacA s genotypes and endoscopic findings.

VacA genotyping		Endoscopic findings			Total
		Non-erosive gastritis	PUD	Gastric malignancy	
S1	No.	5	11	3	19
	%	16.13%	57.89%	100.0%	35.85%
S2	No.	26	8	0	34
	%	83.87%	42.11%	0.0%	64.51%
Total	No	31	19	3	53
	%	100.0%	100.0%	100.0%	100.0%

VIII. VacA m genotype in relation to endoscopic findings (table 11)
There was insignificant association between m1 allele and PUD or gastric malignancy (P=0.81).

Table 11: Relation between VacA m genotype and endoscopic findings.

VacA genotyping		Endoscopic findings			Total
		Non-erosive gastritis	**PUD**	**Gastric malignancy**	
m1	No.	20	10	2	32
	%	64.52%	52.63%	66.67%	60.38%
m2	No.	11	9	1	21
	%	35.48%	47.37%	33.33%	39.62%
Total	No.	31	19	3	53
	%	100.0%	100.0%	100.0%	100.0%
Test	χ^2=0.425 (P=0.81)				

DISCUSSION

Helicobacter pylori (H. pylori) is one of the most common world-wide chronic bacterial infections. H. pylori strains differ, and possession of specific virulence factors greatly increases the risk of the disease. The best recognized of these are CagA and VacA proteins. The present study investigated the relationship between clinically manifest gastroduodenal disease and the genotype of the associated H. pylori organism. According to the demographic data; sex distribution was 55% females and 45% males. The majority of patients were at ages 35-55 years old. A positive correlation between age and prevalence has been reported in both developed and developing countries.[16] Regarding sex distribution, there was insignificant relationship between the gender and the disease status(x^2=0.670, p=0.413).

The endoscopic findings were divided into the following general categories: non erosive gastritis, peptic ulcer diseases (gastric and duodenal) and gastric malignancies (adenocarcinoma and lymphoma). The most common endoscopic finding in our study was non erosive gastritis (n=54, 54%) followed by peptic ulcer diseases (n=39, 39%) and finally gastric malignancies (n=7, 7%).

On the basis of the analysis of expression of these two virulence factors (CagA and VacA).Isolates of H. pylori were divided into three types. Type I (expressing both), type II (expressing neither factor), and type III (CagA expression only). Our results suggested that type I was the most prevalent type (n=53, 53%); the prevalence of type I H. pylori in non-erosive gastritis, peptic ulcer diseases and gastric malignancy was 57.4%(31of 54), 48.7%(19 of 39) and 42.9%(3 of 7) respectively.

As regards the CagA status; of one hundred patients examined in this study, the CagA strain was detected in 94% while most of the controls were CagA –ve (80%). This association was statistically significant (p<0.001). This was similar to many studies reported in Africa and other parts of the world.[17-19]

In Europe and North America, prevalence of CagA positive H. pylori varies between 60% and 79% (e.g. USA, 60%[20]; Spain, 66%([21] and England, 68%[22]), whereas in Asia (e.g. Japan, Korea and China), the proportion of CagA positive H. pylori strains is usually over 90%.[23,24]

In previous studies, strains from Iraq, Turkey and Iran possessing CagA were found in 71%, 78% and 76% of the samples analyzed, respectively.[25] In Jordan, the CagA genotype was detected in 26.4%.[26] While Kuwaitis and other Arabian Gulf Arabs had essentially

the same prevalence rate of about 41%, Egyptians had a modest positivity of 35.7%.[27] In a study conducted in Israel, CagA genes were present in only 25.5%.[28] The prevalence of CagA positivity in Saudi Arabia was 52%.[29]

H pylori CagA positive strains have been associated with more severe gastroduodenal diseases.[30] This study showed a significant positive association between presence of a CagA+ H. pylori strain and non-erosive gastritis ($p<0.001$), peptic ulcer disease ($p<0.001$), and gastric malignancies ($p<0.001$) in agreement with many studies.[31,32]

The VacA is another H. pylori virulence factor.[33] Unlike CagA, almost all H. pylori strains possess the Vacuolating cytotoxin gene. Vacuolating cytotoxin activity is related to the mosaic structure of VacA. In general, type s1/m1 and s1/m2 strains produce high and moderate levels of toxin activity, respectively, whereas s2/m2 strains produce no vacuolating activity. A 12-amino-acid hydrophilic amino-terminal segment, present in type s2 but absent from type s1 VacA proteins, slows the capacity of VacA to form membrane channels and abolishes vacuolation. Heterogeneity among VacA alleles may be an important factor in understanding variations in clinical manifestations among H. pylori-infected subjects.[34]

About half of the cases (53%) in our study were VacA positive (not all the cases) this may be partly due to the presence of different subtypes; not all of them were detected by the primers used.

In our study, the prevalence of VacA s1 genotype was 35.85% (19 of 53 VacA +ve cases), while VacA m1 genotype was 60.38% (32 of 53 VacA +ve cases).

Particular VacA genotypes have been considered markers for the pathogenicity of individual H. pylori strains since production of cytotoxin in vitro, epithelial damage in vivo and the development of peptic ulcer disease are related to specific VacA genotypes. The elevated toxicities of s1 strains may contribute to the development of ulcerations. Indeed, VacA s1 strains secrete larger amounts of cytotoxin than VacA s2 strains in vitro, the latter supposedly being less virulent.[35,36] Indeed, carrying H. pylori with the VacA s1 or s1m1 genotype significantly increased the risk of peptic ulcer or gastric cancer compared with gastritis alone.[37]

Our study revealed that gastric infection with H. pylori strains containing type s1 VacA alleles is associated with a higher risk for development of peptic ulcer disease (OR=7.15, 95% CI=1.91, 26.79,P=0.002) and gastric malignancy (OR=33.73, 95% CI=1.52, 750.35, P=0.010) than infection with strains containing type s2 VacA alleles. Several reports suggested a higher prevalence of the VacA s1 allele in patients with PUD and duodenal ulcers.[38, 39]

Our results showed that there was insignificant relationship (p=0.81) between the VacA m1 genotype and the development of PUD or gastric malignancy. Studies from Iraq, Kuwait, Jordan, Israel and Iran did not show any association between VacA s and m genotypes and gastroduodenal diseases.[40,41]

CONCLUSION

Our study showed that CagA and VacA s1 variant are associated with more severe disease outcome in Egypt. In addition, we demonstrated that the relatively low percentage of VacA s1 allele in Egypt may be one of the causes of the low prevalence of gastric cancer despite high prevalence of CagA in H.pylori infected Egyptian patients.

REFRENCES

1. Rust M, Schweinitizer T, and Josenhans C. Helicobacter Flagella, Motility and Chemostaxis. Helicobacter pylori: Molecular Genetics and Cellular Biology (Yamaoka Y, ed). Caister Academic Press 2008; ISBN1-904455-31-8.
2. Lehours Pand Yilmaz O. Epidemiology of Helicobacter pylori infection. Helicobacter, 2007; 12: 1-3.
3. Montecucco Cand Rappuoli R. Living dangerously: how Helicobacter pylori survives in human stomach. Nat Rev Mol Cell Biol., 2001; 2: 457-66.
4. Kindermann A and Lopes AI. Helicobacter pylori infection in pediatrics. Helicobacter, 2009; 14: 52-7.
5. Yamaoka Y. Roles of the plasticity regions of Helicobacter pylori in gastroduodenal pathogenesis. J med Microbiol, 2008; 57: 545-53.
6. Peek RM Jr and Blaser MJ.Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer, 2002; 2: 28-37.
7. Peek RM Jr, Moss SF, Tham KT, Perez-Perez GI, Wang S, et al. Helicobacter pylori CagA +ve strains and dissociation of gastric epithelial cell proliferation from apoptosis. J Natl Cancer Inst, 1997; 89: 863-8.
8. Ameiva MR, Vogelmann R, CoVacci A, Tompkins LS, Nelson WJ, et al. Disruption of the epithelial apical- junctional complex by Helicobacter pylori CagA. Science, 2003; 300: 1430-4.
9. Argent RH, Kidd M, Owen RJ, Thomas RJ, Limb MC, et al. Determinants and consequences of different levels of Cag A phosphorylation for clinical isolates of Helicobacter pylori. Gatroenterology, 2004; 127: 514-23.
10. Atherton JC, Cao P, Peek RM, Tummuru MKR, Blaser MJ, et al. Mosaicism in Vacuolating cytotoxin alleles of Helicobacter pylori –association of specific VacA types with cytotoxin production and peptic ulceration. J Biol Chem., 1995; 270: 17771-7.
11. Letley DP, Rhead JL, Twells RJ, Dove B, Atherton JC. Determinants of non-toxicity in the gastric pathogen Helicobacter pylori. J Biol Chem., 2003; 278: 26734-41.

12. Rhead JL, Letley DP, Mohammadi M, et al. A new Helicobacter pylori Vacuolating cytotoxin determinant, the intermediate region is associated with gastric cancer. Gastroenterology, 2007; 133: 926-36.
13. Veijola L, Myllyluoma E, Korpela R, and Rautelin H. Stool antigen test in the diagnosis of helicobacter pylori infection before and after eradication therapy. World J Gastroenterol, 2005; 11: 7340-4.
14. Vaira D, Gatta L, Ricci C, Miglioli M. Diagnosis of helicobacter pylori infection. Alimentary pharmacology & therapeutics, 2002; 16: 16-23.
15. Kabir S. Detection of helicobacter pylori DNA in faeces and saliva by polymerase chain reaction: a review. Helicobacter, 2004; 9: 115-23.
16. Boixeda D, Martin DC, Argila C, and Canton R. Prevalence of H.pylori in symptomatic adults. Rew Esp Engerm Dig., 1994; 86: 569-76.
17. Wong BCY, Yin Y, Berg DE, Xia H, Zhang JZ, Wang WH, et al. Distribution of distinct VacA, CagA , and ice A alleles in Helicobacter pylori in Hong Kong. Helicobacter, 2001; 6: 317-24.
18. Smith SI, Kirsh C, Oyedeji KS, Arigbabu AO, Coker AO, Bayerdoffer E, et al. Prevalence of Helicobacter pylori VacA, CagA and ice A in Nigerian patients with duodenal ulcer disease. J Med Microbiol, 2002; 51: 851-4.
19. Asrat D, Nilsson I, Mengistu Y, Ashenafi S, Ayenew K, Al-Soud AW, et al. Prevalence of Helicobacter pylori VacA and CagA genotypes in Ethiopian dyspeptic patients. J Clin Microbiol, 2004; 42: 2682-4.
20. Tummuru MK, Cover TL, and Blaser MJ. Cloning and expression of a high-molecular-mass major antigen of Helicobacter pylori: evidence of linkage to cytotoxin production. Infect Immun, 1993; 61: 1799-809.
21. Alarcón T, Domingo D, Martinez MJ, and López-Brea M. CagA gene and VacA alleles in Spanish Helicobacter pylori clinical isolates from patients of different ages. FEMS Immunol Med Microbiol, 1999; 24: 215-9.
22. Warburton VJ, Everett S, Mapstone NP, Axon AT, Hawkey P, and Dixon MF. Clinical and histological associations of CagA and VacA genotypes in Helicobacter pylori gastritis. J Clin Pathol 1998; 51: 55-61.
23. Chen XJ, Yan J, and Shen YF. Dominant CagA/VacA genotypes and coinfection frequency of H. pylori in peptic ulcer or chronic gastritis patients in Zhejiang Province and correlations among different genotypes, coinfection and severity of the diseases. Chin Med J (Engl), 2005; 118: 460-6.
24. Erzin Y, Koskal V, Altun S, Dobrucali A, Aslan M, Erdamar S, et al. Prevalence of H. pylori VacA, CagA, CagE, iceA, and babA2 genotypes and correlations with clinical outcomes in Turkish patients with dyspepsia. Helicobacter, 2006; 11: 574-80.
25. Saribasak H, Salih BA, Yamaoka Y, and Sander E. Analysis of Helicobacter pylori genotypes and correlation with clinical outcome in Turkey. J Clin Microbiol, 2004; 42: 1648-51.
26. Nimri LF, Matalka I, Bani Hani K, and Ibrahim M. Helicobacter pylori genotypes identified in gastric biopsy specimens from Jordanian patients. BMC Gastroenterol, 2006; 6: 27.
27. Al Qabandi A, Mustafa AS, Siddique I, Khajah AK, Madda JP, and Junaid TA. Distribution of VacA and CagA genotypes of Helicobacter pylori in Kuwait. Acta Trop, 2005; 93: 283-8.
28. Benenson S, Halle D, Rudensky B, Faber J, Schlesinger Y, Branski D, et al. Helicobacter pylori genotypes in Israeli children: the significance of geography. J Pediatr Gastroenterol Nutr, 2002; 35: 680-4.
29. Momenah AM, and Tayeb MT. Helicobacter pylori CagA and iceA genotypes status and risk of peptic ulcer in Saudi patients. Saudi Med J., 2007; 28: 382-5. 190.
30. Hatakeyama M, and Higashi H. Helicobacter pylori CagA: new paradigm for bacterial carcinogenesis. Cancer Sci., 2005; 96: 835-43.
31. Blaser MJ, and Atherton JC. Helicobacter pylori persistence: biology and disease. J Clin Invest, 2004; 113: 321-33.
32. Palli D, Masala G, Del Giudice G, et al. CagA Helicobacter pylori infection and gastric cancer risk in the EPIC-EURGAST study. Int J Cancer, 2007; 120: 859-67.
33. Atherton JC. H. pylori virulence factors. Br Med Bull., 1998; 54: 105-120.
34. Atherton JC, Cao P, Peek RM, Tummuru MKR, Blaser MJ, et al. Mosaicism in Vacuolating cytotoxin alleles of Helicobacter pylori—association of specific VacA types with cytotoxin production and peptic ulceration. J Biol Chem., 1995; 270: 17771-7.
35. Atherton JC, Peek RM, Tham KT, Cover TL, and Blaser MJ. Clinical and pathological importance of heterogeneity in VacA, the Vacuolating cytotoxin gene of Helicobacter pylori. Gastroenterology, 1997; 112: 92-99.
36. Van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, and de Boer W. Clinical relevance of the CagA, VacA, and iceA status of Helicobacter pylori. Gastroenterology, 1998; 115: 58-66.
37. Sugimoto M, and Yamaoka Y. The association of VacA genotype and Helicobacter pylori-related disease in Latin American and African populations. Clin Microbiol Infect, 2009; 15: 835-42.
38. Van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, and de Boer W. Clinical relevance of the CagA, VacA, and iceA status of Helicobacter pylori. Gastroenterology, 1998; 115: 58-66.
39. Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, and Graham DY. Relationship between Helicobacter pylori iceA, CagA, and VacA status

and clinical outcome: studies in four different countries. J Clin Microbiol, 1999; 37: 2274-9.

40. Hussein NR, Mohammadi M, Talebkhan Y, Doraghi M, Letley DP, Muhammad MK, et al. Differences in Virulence Markers between Helicobacter pylori Strains from Iraq and Those from Iran: Potential Importance of Regional Differences in H. pylori-Associated Disease. Journal of Clinical Microbiology, 2008; 46: 1774-9.
41. Benenson S, Halle D, Rudensky B, Faber J, Schlesinger Y, Branski D, et al. Helicobacter pylori genotypes in Israeli children: the significance of geography. J Pediatr Gastroenterol Nutr, 2002; 35: 680-4.